0.36 in HCC827 and p = 0.45 in H4006). PD-1 expression of CD8 T cell cocultured with HCC827 or H4006 did not change; however, proportion of regulatory T cell increased after coculture with HCC827 or H4006 (p=0.05 and p=0.08, respectively) and did not decrease during EGFR-TKI treatment. Proportion of regulatory T cell in cocultures with A549 or H1975 (erlotinib resistant cell line) did not change during coculture or EGFR-TKI treatment. Increase of IP-10 is mediated by IFN-γ in both EGFR mutant cell lines and PBMCs. The inhibition of IP-10 by si-RNA significantly decreased TGF-β1 expression and proportion of regulatory T cells in cocultured mutant EGFR lung cancer cell with EGFR-TKI treatment. Transcriptome analysis by RNA sequencing showed 1,747 gene sets were differentially expressed in EGFRTKI treated EGFR mutant cell line cocultured with activated PBMC compared to EGFR-TKI treatment alone. Interferon gamma response pathway (NES 2.65, FDR q < 0.36) was the most significantly changed. Immune profile analysis of human EGFR mutant lung cancer showed marked heterogeneity in total lymphocyte infiltration, as low as 8.03% or as high as 44.7% of live cells. Among immune cells, proportion of CD4+CD3+ T cells in EGFR mutant groups was increased compared to EGFR wild group (62.7 ± 2.96 vs. 55.14 ± 5.1% among CD3+ T cells) and proportion of FOXP3+CD25+CD4+ Treg in EGFR mutant group tended to increase compared to EGFR wild group (1.352 ± 0.4 vs. 0.74 ± 0.16%, p=0.256). Conclusions: The increased regulatory T cell by IP-10 and TGF-β is considered to be important in EGFR mutant NSCLC in immune-suppressive microenvironment and EGFR-TKI resistance.

A30
Tumor-Infiltrating Lymphocytes (TILs) Found Elevated in Lung Adenocarcinomas (LUAD) Using Automated Digital Pathology Masks Derived from Deep-Learning Models


Background: Tumor mutation burden (TMB) is associated with increased response to anti-PD-1 therapy in non-small cell lung cancer (NSCLC) (Rizvi, 2015). Squamous cell carcinomas (LUSC) have higher average TMB than adenocarcinomas (LUAD) (Schumacher, Schreiber, 2015); however, meta-analyses show that in fact LUAD receive slightly more survival benefit from anti-PD1 therapy (Zhou, 2018). Here we explored whether lymphocyte distribution in the tumor microenvironment may give a rational explanation for this differential response to immune-oncology (IO) agents.

Methods: 867 subtype NSCLC high-resolution diagnostic whole-slide images were obtained from TCGA sources. Images were tiled into 100micron 2D color patches. To ensure subtypes were visually distinct at this scale, a LUAD/LUSC classifier was developed as follows: Samples were randomly split into 80% training and 20% testing samples. Cells were counted in each image patch, and used to bin into 12 ranges of cell counts (0-10 cells per patch, 10-20, etc., up to >110 cells per patch). 2D color patches were transformed into 1D descriptive vectors using the ResNet54 deep learning framework, and used to train 12 separate support-vector machines (SVMs). An ensemble of these 12 SVMs was used to classify unseen samples. To detect tumor regions and lymphocyte infiltration, 2D color patches were used to train convolutional neural networks (InceptionV3) based on gold-standard masks generated with pathology assistance, then used to detect tumor and lymphocytes in all unseen patches. Patches that simultaneously classified as positive for tumor and lymphocyte area were considered evidence of TILs. Lymphocyte-positive patches immediately adjacent to tumor patches (i.e., lymphocytes within 100microns of tumor) were also analyzed. Results: LUAD and LUSC were highly classifiable using this system, with a ROC AUC of 0.95 and precision of 0.95 in test samples. The total tumor tissue area was similar between samples classified as LUAD and LUSC (48.3%/+-1.1% vs. 46.5%/+-1.1%). Whole-slide lymphocyte level was similar although slightly lower in LUAD (9.9%/+-0.2% vs. 11.4%/+-0.2%). However, lymphocytes in LUAD samples were more likely to infiltrate tumor regions than those in LUSC (48.1%/+-1.2% vs. 42.7%/+-0.7%), and/or were immediately adjacent to tumor regions (78%/+-1.2% vs. 74.2%/+-0.9%). Lymphocyte levels were more bimodal in LUAD than LUSC, with 28.6% (vs. 22.9%) having very high TIL (>60%) despite having lower overall lymphocyte counts. Conclusions: Despite lower overall TMB and lymphocyte levels, there exists a subset of LUAD samples with very high infiltrating lymphocyte counts, indicating a potentially anti-PD1-sensitive subpopulation. Further characterizing this subset and confirming differential IO response is warranted.

A31
A Reservoir of Tumor-Specific CD8 T Cells in Lung Cancer Resides in the Draining Lymph Node

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Recent work has described the population of CD8 T cells that respond to anti-PD-1 therapy (marked by TCF1 and PD-1), but it remains unclear what these CD8 T cells are maintaining within the immunosuppressive tumor microenvironment (TME) of lung cancer. To understand this, we developed a genetically engineered model in which Kras-G12D expressing p53 deficient lung adenocarcinomas express a known neoantigen called the iNversin Induced neoAntigen (NINJA). NINJA allows us to follow neoantigen-specific CD8 T cells over the course of tumor development. We find that ~20% of tumor-specific T cells in early 8-wk tumors are TCF1+, but by 17-20 wks, this TCF1+ has significantly shrunk, and there has been a concomitant increase in the expression of markers of T-cell terminal differentiation (Tim3). This is consistent with the idea that T cells receive signals in the TME that drive terminal differentiation and restrict responses to immunotherapy. We reasoned that if the signals driving terminal differentiation were provided in the TME, neoantigen-specific T cells in the tumor-draining lymph node (dLN) may remain less differentiated over the course of tumor development. Analysis of tumor-specific T cells in the dLNs of 8-wk and 17-wk tumors showed that they were mostly TCF1+. Moreover, single-cell transcriptional analyses suggested that these cells were less differentiated than their counterparts in tumors. T-cell receptor (TCR) signals are a major driver of terminal differentiation, and we observed that tumor-specific T cells in the dLN were not receiving TCR signals, while all T cells in the TME were positive for TCR signals. This suggested at least two models for how antitumor T cells function: 1) tumor-reactive T cells in dLNs and TME could function independently of one another, or 2) tumor-reactive T cells might have a role in sustaining the antitumor T-cell response over the course of tumor development through migration. Consistent with the latter model, TCR sequencing of dLN and TME neoantigen-specific T cells showed a close clonal relationship: 13 of the top 15 clones in the TME were present in the dLN, and the hierarchy of clonal dominance was maintained. This was also true in 17-wk tumor-bearing mice, suggesting that the population of tumor-specific CD8 T cells in the dLN serves as a reservoir of less differentiated cells that can continuously replenish the T cells in the TME, helping to sustain the antitumor T-cell response over the course of tumor development. Critically, it is unclear whether current immunotherapy-appefect strategies leverage this reservoir of T cells, raising the possibility that this population of dLN tumor-specific T cells could be targeted to provide significant additional benefit for patients receiving immunotherapy.